EXPERIMENTS ON THE USE OF *BACILLUS PESTIS-CAVIAE* AS A RAT VIRUS.*

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THE following details are drawn from notes kept on a series of experiments conducted at the Federal Laboratory between April 15 and November, 1908. The experiments with a commercial ratticide known as "Azoa" were undertaken at the request of Passed Assistant Surgeon J. D. Long, then in charge of the plague situation in Oakland, Cal., and at the request of the representatives of Parke, Davis & Co., who had asked for a fair experimental trial of their virus. This and subsequent experimental work was prosecuted with the aid of federal money, obtained by Passed Assistant Surgeon Rupert Blue, commanding.

EXPERIMENTS WITH A COMMERCIAL VIRUS KNOWN AS AZOA.

Series I.—The following feeding experiment as detailed below was commenced before my arrival in Oakland. I was informed that the 12 rats (*M. norvegicus*) fed on azoa received the virus on the dates indicated below, each feeding for the 12 consisting of 4 oz. (124 gms.) of azoa, lot No. 010916–E, thoroughly mixed with 32 oz. (992 gms.) of dry rolled oats.

Four of the rats set aside for controls died before the feeding was commenced, probably from arsenical poisoning. All the rats were fed on rolled barley, daily, excepting Sundays when they were not fed at all, and excepting one feeding of raw meat (date not given) and excepting the dates when 12 of the rats were fed azoa.

Ten of the rats which died after feeding on azoa were examined 12–18 hours after death. Only four of these showed noticeable abnormality, some enlargement of the spleen and liver with numerous yellowish-white foci of necrosis scattered beneath the capsule of these organs. Microscopic examinations failed to reveal anything noteworthy excepting the rare occurrence of a bipolar staining diplobacillus in liver and spleen smears. Cultures from the spleens of these rats,

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TABLE 1.

AZOA FED RATS.

No.	Date Caught	Feedings			Number of Days after Last Feeding till Death
		1st	2d	3d	
1	April 8	April 9	April 11	April 15	9
2	" 8	" 9	" 11	" 15	9
3	" 8	" 9	" 11	" 15	10
4	" 8	" 9	" 11	" 15	6
5	" 8	" 9	" 11	" 15	8
6	" 8	" 9	" 11	" 15	8
7	" 7	" 8	" 11	" 15	
8	" 7	" 8	" 11	" 15	8
9	" 7	" 8	" 11	" 15	6
10	" 7	" 8	" 11	" 15	8
11	" 7	" 8	" 11	" 15	5
12	" 8	" 9	" 11	" 15	6

CONTROL RATS.

No.	Date Caught	Date Died	Cause of Death	Still Alive
1	April 7	April 12	Probably arsenic	
2	" 7	" 12	" "	
3	" 8	" 12	" "	
4	" 8			
5	" 7			April 27
6	" 7			" 27
7	" 8	" 14	Not determined	
8	" 8			" 27
9	" 8	" 15	" "	
10	" 7			" 27
11	" 8			" 27
12	" 7			" 27

however, uniformly yielded, in 24 hours, from 20 to 50 similar ring-like colonies. As will be seen below, they all died of infection with *B. pestis-caviae*. The six remaining controls were chloroformed at the close of the experiment and cultures from their spleens remained sterile.

In order to check the results already obtained and to prove whether the disease was transmissible by feeding rats on the organs of rats supposedly dead from azoa, another series was started.

Series II.—On May 30, 35 adult *M. norvegicus* were collected, and each rat was placed in a separate cage-trap supplied with firmly secured feed and water pans. They were numbered from 13 to and including 47. The traps were then arranged on a sawdust bed in a room sealed and locked with a yale lock. During the first four days, eight of the rats died, Nos. 13, 14, 15, 17, 18, 23, 28, and 31. Rat 18 had the leprosy-like disease and No. 31 died of an infection due to streptococci appearing as diplococci in the tissues. Rat 28 had a

yellow liver probably due to arsenic. The cause of death was not ascertained in the other instances. All of the remaining rats were fed on cracked barley one day and on cabbage leaves the next for a week, and were watered every day. As they now all appeared healthy and lively, they were separated into two lots of 12 each; 12 azoa rats, and 12 controls. They were all starved one day, and then the azoa rats received their first feeding of 4 oz. (124 gms.) of azoa, lot No. 010916–E, thoroughly mixed with 32 oz. (992 gms.) of dry rolled oats. The 12 controls were fed on 992 gms. of dry rolled oats.

The details of these feedings by dates are as follows:

1st feeding, June 8, Fed most of the azoa mixture to the azoa rats; watered.
Fed most of the plain oatmeal to the control rats; watered.
June 9, Fed rest of the azoa mixture to the azoa rats; watered.
Fed rest of the plain oatmeal to the control rats; watered.
June 10, Starved all the rats; watered.
2d feeding, June 11, and 12, Repeated feeding as per 1st feeding; watered.
June 13, All rats fed on cabbage and watered.
June 14, All rats starved.
3d feeding, June 15, and 16, Repeated feeding as per 1st feeding; watered.

The rats of this series were fed rolled oats one day and cabbage the next and watered daily. Excepting one, which had a bad case of scabies of the ears and neck, and rat 20 which died on the twentieth day after the last feeding, they all remained in perfect health. Rat 20 showed no anatomical changes but agar slants from its spleen showed numerous colonies which were proven to be those of *B. pestis-caviae*. They grew fat for over two months when they were chloroformed and their spleens proven sterile culturally.

Having in mind the work of Weiske on the oxalic acid poisoning of rabbits kept on a diet of oats alone, and the more recent work of Holst and Frolich[1] on the toxic action of a single diet on guinea-pigs, I started to determine whether experimental rats kept on a barley or oats diet alone might either die of a scurvy-like disease or become susceptible to invasion with a micro-organism, whereas when fed cabbage (which has been shown to neutralize the toxic action of oats, barley, etc.) they might survive. It was with this possibility in view that cabbage was introduced with the diet in Series II. However, experiments showed that rats, *M. norvegicus*, are not injured

[1] *Jour. Hyg.*, 1907, 5, p. 663.

in the least by being kept on a single diet of oats and barley. For example, six adult rats were fed daily on barley and water for two months. They grew fat and sleek and showed no abnormalities when chloroformed and carefully dissected.

In another series six rats were fed on azoa, according to the plan detailed under Series II, and then kept on a barley-and-water diet for 20 days, when they were chloroformed. They all appeared normal at necropsy and cultures from their spleens remained sterile.

Again four rats were kept on a diet of rolled oats and water for over a month with apparent benefit.

B. pestis-caviae AND ITS RELATION TO THE VIRUS OF AZOA.

During the course of these experiments, I encountered a pseudotuberculous disease in my stock of guinea-pigs. The only lesions of this disease I have encountered are the occurrence of a few or many firm, yellowish-white nodules in the spleen. These vary from less than 1 to 5 or 6 mm. in diameter. On section, they contain a semipurulent or caseous material. Smears from these nodules show considerable numbers of rods with rounded ends. When fixed by heat and stained with carbol-thionin most of them show excellent bipolar staining. When measured (Zeiss $\frac{1}{12}$ oc. microm. 6) they are most frequently $1.8 \times 0.8\ \mu$ though smaller coccoid forms $0.8 \times 0.8\ \mu$ and longer forms up to $2.8 \times 0.8\ \mu$ may be seen. They do not retain the stain in Gram's method.

As will be detailed below cultural studies, agglutination, and animal experiments show that the virus of azoa is identical with that of this pseudotuberculous disease of guinea-pigs. (It is highly improbable that these stock guinea-pigs obtained their infection from azoa, for they were kept in a separate building from the one in which the azoa experiments were conducted. The dealer from whom the guinea-pigs were obtained lived on the outskirts of the city and had raised them himself. He had never used a rat virus about his premises.)

This disease has been known since 1896. It was first described by Theobald Smith and J. R. Stewart.[1] In a later reference[2] it is described by Dr. Smith as "a bacillus isolated from a guinea-pig in 1896, and then called pseudotuberculosis, owing to the presence of

[1] *Jour. Boston Soc. Med. Sci.*, 1897, p. 12.

[2] Smith and Reagh, *Jour. Med. Res.*, 1903, 9, p. 273.

large tubercle-like foci in the liver and spleen which were necrotic-suppurative in character. This is evidently the same as the bacillus isolated by Dr. Edward P. Carter at Johns Hopkins University in 1897, a culture of which was kindly sent to me by Dr. Carter, and another recently by Dr. Harris, for comparison with our bacillus. As Dr. Carter did not publish his studies upon this organism, we may state here that in a personal communication Dr. Carter called it the bacillus of infectious endometritis in guinea-pigs. It was isolated from a spontaneous epizootic in which the females chiefly were carried off by it. They always showed an acute endometritis. A study of our bacillus and of Dr. Carter's bacillus showed no appreciable differences." Dr. Smith tells me that the Rockefeller Institute recently lost its entire stock of guinea-pigs from this disease.

So far as is known the original host of the germ seems to be the guinea-pig (*Cavia Aperea*). I have called it *Bacillus cholera-caviae* in a preliminary note[1] but on the recommendation of Dr. Smith it has been changed to *Bacillus pestis-caviae*, "since the disease may appear as multiple spleen and liver abscesses or as a puerperal disease."

Biochemical Characters of *B. pestis-caviae.*

Cultural characters.—When plated in agar[2] or litmus-lactose agar, circular, white, slightly elevated colonies, punctuate to 0.5 mm. to 1 mm., appear in 24 hours at 37° C. When well separated they may spread out to 3 or 4 mm. in diameter. They appear light brownish by transmitted light and produce no change in lactose. On stroke cultures made on agar slants from the tissues of infected animals the colonies are of the same character when well separated. If very numerous and closely aggregated they appear as minute transparent colonies. The growth is not viscous. Quite frequently the growth from animal tissues appears in the form of "ring-colonies" the bacteria being piled up in a white ring about a clear central space.[3] The organism produces alkali rapidly and this fact may be taken advan-

[1] *Public Health Reports*, November, 1908.

[2] Unless otherwise mentioned the reaction of the media was (+1) 1 per cent acid to phenolphthalein.

[3] This phenomenon has been noted before by Theobald Smith and Reed and Carroll (*Jour. Exper. Med.*, 1900, 5, p. 233) in the case of the colonies of *B. cholera-suum* and *B. icteroides* and I have noted it in the colonies of *B. coli* inoculated directly from the tissues of ground squirrels and humans.

tage of when it is mixed with acid producers on litmus-lactose-agar plates.

Sugar-free broth is evenly clouded and a faint but distinct indol reaction can be obtained after 10 days' growth at 37° C. In young cultures the bacilli are quite as actively motile as typhoid bacilli. In old broth cultures rather coarse stalactites may be produced.

There is good growth in +1.5 gelatin at 25–28° C. without any liquefaction during two months' observation. There is no visible change in milk cultures for several days but in 7 or 10 days at 37° C. the culture appears opalescent, and later it is turned to a clear, yellowish fluid.

Dextrose, levulose, maltose, mannite, and galactose are fermented with gas production ($H/CO_2 = 2/1$). No acid nor gas is produced from lactose, saccharose, nor inulin. As in the case of many bacteria, growth is much more luxuriant when a fermentable carbohydrate is present.

In order to determine the systematic position of *B. pestis-caviae* and its relationship to the virus of azoa, a careful comparative study was made of the following cultures: from the spleens of rats 2, 5, 7, and 9, which died in the azoa feeding experiment (Series I); from the spleen of rat 12, which died from a subcutaneous inoculation with an emulsion of azoa; from the spleen of rat 20 (feeding experiment Series II), a culture isolated from azoa itself by plating in litmus lactose agar; from the pseudotuberculous spleen nodules of two guinea-pigs (July 8 and July 13); a culture of Danysz virus for rats, brought by me from Manila where I had myself transplanted it from the original tube from the Kràl Laboratory; a strain of what appeared to be *B. enteritidis* isolated by myself from the liver of human case 14; and a strain of the hog-cholera bacillus obtained from the Hygienic Laboratory, Washington. All of these cultures correspond in every detail as above described for *B. pestis-caviae* excepting the lack of indol production in the case of *B. cholera-suum.*

Rabbits were immunized for a series of inter-agglutination relattionship tests but I have had time only to complete one of the series; a Belgian hare was immunized with the culture from the spleen of rat 2 which died in the azoa feeding experiment (Series I). In all, 11 c.c. of broth cultures killed at 60° C. were injected during a

month and a half. The cultures to be tested were grown for 24 hours in sugar-free broth, brought up to as nearly a uniform density as could be determined with the naked eye, and 0.5 c.c. of each culture mixed with an equal amount of the diluted serum and kept at 37° C. for one hour. The following table gives the results of the first test with cultures; spleen azoa rat 2 (Series I); spleen azoa rat 20 (Series II); *B. pestis-caviae* (spleen guinea-pig of July 13); Danysz bacillus; and hog cholera.

TABLE 2.

Cultures	Dilutions of Serum					
	$\frac{1}{10}$	$\frac{1}{20}$	$\frac{1}{40}$	$\frac{1}{80}$	$\frac{1}{160}$	$\frac{1}{320}$
2	++	++	+	+	+	+
Controls	−	−	−	−	−	−
20	++	++	+	+	+	+
Controls	−	−	−	−	−	−
Guinea-pig, July 13	++	++	+	+	+	+
Controls	−	−	−	−	−	−
Danysz bacillus	+	+	±	−	−	−
Controls	−	−	−	−	−	−
Hog cholera	++	++	+	±	−	−
Controls	+	+	+	±	−	−

+++ = complete precipitation.
++ = almost complete.
+ = many flocculi in suspension.
± = few flocculi in suspension.
− = remains uniformly turbid.

The presence of normal agglutinins, in the serum of Belgian hares, for the hog-cholera bacillus does not nearly equal the amount present for the typhoid bacillus. However, as they appeared to be present in sufficient quantity to obscure the value of the comparative test so far as the hog-cholera bacillus was concerned, the rabbit was given another immunizing dose and another series of tests made in the manner detailed above, excepting that the readings were taken after two hours at 37° C. and the culture from the liver of H.14 substituted for that of rat 20. The results of this test show not only that the culture from azoa rat 2 and that from the pseudotuberculous disease of guinea-pigs are identical but that they are probably more closely related to *B. enteritidis* H.14 than to hog-cholera or to Danysz bacillus, though Danysz bacillus is said to be identical with *B. enteritidis* (Gärtner).

TABLE 3.

CULTURES	DILUTIONS OF SERUM						
	1/200	1/400	1/600	1/800	1/1000	1/2000	1/4000
Azoa rat 2.....	+++	++	++	+	±	±	±
Guinea-pig, July 13.	+++	++	++	+	±	±	±
Liver, Human 14...	±	−	−	−	−	−	−
Danysz.....	−	−	−	−	−	−	−
Hog cholera.....	−	−	−	−	−	−	−

PATHOGENICITY OF *B. pestis-caviae.*

Guinea-pigs.—When guinea-pigs of 300–500 gms. weight are injected subcutaneously with 2 c.c. of a 24-hour-old sugar-free broth culture they die in three to six days. On dissection the skin of the abdomen is firmly bound to the muscular wall by a hemorrhagic fibrinous exudate, which is of a purulent nature at the point of inoculation. A sero-gelatinous exudate may occur in the groins. The spleen is enlarged and soft when the animal lives for five or six days. Hydrothorax and fibrino-purulent pericarditis may be found. The other internal organs are generally congested. Numerous bipolar staining bacilli often in pairs occur in the subcutis or pericardial exudate. A few bipolar-staining diplobacilli may be found in spleen or liver smears. Cultures show that there are numerous bacilli in the spleen even when they appear scarce microscopically. Passage from guinea-pig to guinea-pig produces a marked increase in the severity of the local reaction but does not materially shorten the course of the infection during 11 or 12 passages as detailed further on in Table 4. Doses of 0.25–0.75 c.c. usually produce a localized abscess followed by necrosis, sloughing, and recovery. Intraperitoneal injection produces death more rapidly with marked fibrino-purulent peritonitis and septicemia.

Rats (*M. norvegicus*).—Four c.c. of a 24-hour-old sugar-free broth culture must be injected subcutaneously into adult rats (150–350 gms.) in order to produce any uniform lethal effects. When they succumb, the average time appears to be about 8–12 days. On dissection there is local abscess production. The spleen is enlarged, sometimes to four or five times the normal size, soft, and usually shows numerous yellowish-white specks of necrosis. If the rat survives and is chloroformed, minute yellowish-white nodules, 0.5–

2 mm. in size may be found in this organ. The liver may simply appear congested or also show numerous yellowish-white foci of necrosis. Occasionally there are dark coffee-colored areas of hemorrhage in the lungs; also sometimes hydrothorax. The lymph glands do not appear to be involved and I have not seen the secondary pericarditis which sometimes occurs in guinea-pigs.

When ingested, a large percentage of adult rats are immune, though they occasionally die with the above-mentioned anatomical changes or simply with congestion and swelling of the spleen and liver. Young rats (50–150 gms.) and mice (*M. musculus*) are quite uniformly susceptible to infection by feeding. They either succumb rapidly to an acute septicemia without any marked anatomical changes or, when more resistant, they show the anatomical changes in the spleen and liver, and sometimes hemorrhages in the lungs, as described above. Rats infected with this virus do not appear to be ill until shortly before their death. I have on a few occasions watched them die. They lie with legs extended and exhibit embarrassed respiration. The respirations gradually become slower and shallower until death ensues.

Looking back over the feeding experiments with azoa already detailed, this factor of age will explain the difference in the results obtained in Series I and Series II.

Rabbits.—A rabbit weighing 1,160 gms. was injected subcutaneously with 1 c.c. of a 24-hour-old broth culture. A localized abscess was produced. This ruptured and healed.

I have not found time to test the possible pathogenicity of *B. pestis-caviae* for the various domestic animals. This has apparently been done by Parke, Davis & Co.

A single feeding-experiment with a monkey will be detailed. An adult Rhesus monkey was starved 12 hours and then fed on 30 c.c. of a 24-hour-old sugar-free broth culture, inoculated directly from the spleen of rat 18 (Table 5) mixed with boiled rice and chopped apple. It ate the mixture greedily and has remained well up to date, a period of one month. The ground squirrel of this region, *O. beecheyi*, is immune to infection by feeding on large doses of sugar-free broth cultures.

Various attempts have been made to raise the virulence of this

organism and these will be detailed here in connection with the general subject of pathogenicity.

EXPERIMENTS ATTEMPTING TO RAISE THE VIRULENCE BY PASSAGE FROM RAT TO RAT BY INOCULATION AND FEEDING.

In this series adult rats (*M. norvegicus*) over 150 gms. were used throughout. The original culture of *B. pestis-caviae* from the spleen of the guinea-pig of July 13 was used to start the series on September 2, and all subsequent experimental work has been conducted with this stock or its subcultures. Preliminary experiments showed that it was necessary to use 4 c.c. of a sugar-free broth culture grown for 24 hours at 37° C. in order to get any uniformly lethal effects when injected subcutaneously into adult rats. Occasionally death was produced by 2 or 3 c.c. In order to shorten the time the organism must spend outside of an animal host, 10 c.c. sugar-free broth tubes were inoculated directly from the spleen of one victim and then after 24 hours' growth 4 c.c. were inoculated subcutaneously into another. These fluid cultures were controlled for purity as far as practicable by the examination of hanging-drop preparations and control cultures on agar slants. Occasionally dextrose, lactose, and saccharose tubes were inoculated as controls. Out of four series of such passages I will cite one:

Table 5 records the subcutaneous passages through a series of adult rats. The culture with which the first rat was injected was derived from the spleen of a rat which died on the tenth day after feeding on 7 c.c. of a 24-hour-old milk culture of *B. pestis-caviae*. The results showed at least that no rapid gain in virulence could be obtained by this method.

TABLE 5.

Rat	Days till Death	Chloroformed in Days	Organism Recovered in Cultures
2.....	8	..	++
9.....	8	..	+
13.....	8	..	+++
18.....	8	..	+++
32.....	..	20	−

+++ = Numerous colonies, spleen, liver, and heart's blood.
++ = Numerous colonies, spleen only.
+ = Few colonies, spleen.

Table 6 illustrates another series in which passages by inoculation

and feeding were combined. This series was started as indicated above, Table 5. When rat 2 died its spleen and liver were fed to rat 13 and likewise the spleen and liver of rat 13 to rat 14, and cultures from the spleen of rat 14 inoculated into rats 25, 26, 27 with negative results:

TABLE 6.

Rat	Days till Death	Chloroformed in Days	Organism Recovered in Cultures
2.....	8	..	++
13.....	8	..	+++
14.....	16	..	+++
25, 26, 27.....	..	21	−

INOCULATION AND FEEDING EXPERIMENTS FOLLOWING THE ATTEMPT TO RAISE THE VIRULENCE OF *B. pestis-caviae* BY PASSAGE THROUGH GUINEA-PIGS.

Experiments showed that about 2 c.c. of a 24-hour-old sugar-free broth culture was necessary to kill guinea-pigs of 300–500 gms. when injected subcutaneously. The culture fluid used for passage was inoculated directly from the spleen of a dead guinea-pig. The passages can be divided into two series. (Table 7.)

TABLE 7.

Series I				Series II			
Guinea-pig Number	Weight Gms.	Dose Subcutaneously in c.c.	Days before Death	Guinea-pig Number	Weight Gms.	Dose Subcutaneously in c.c.	Days before Death
1.....	460	2 (intraperitoneal)	1	1.....	460	2 (intraperitoneal)	1
2.....	475	2	5	2.....	475	2	5
4.....	450	2	3	4.....	450	2	3
5.....	471	2	4	6.....	455	1	7
7.....	514	2	6	9.....	343	0.5	6
11 and 12....	370 398	2	4	14.....	390	1	6
16.....	331	1.5	3	18.....	290	1	4
17.....	300	1.5	2	21.....	465	1	9
19.....	310	1	2	24.....	500	3	3
20.....	530	1.5	3	25.....	420	2	3
22.....	466	0.75	abscess recovered	26.....	435	2	3
				27.....	458	2	6

There was evidently no material increase in the virulence of the organism for guinea-pigs in 11 or 12 such passages. An adult rat was fed on the spleen, liver, and a piece of the local reaction of guinea-pig 12 (Series I, Table 7). It died in six days. The cultures from its spleen were passed through four series of rats by subcutaneous

inoculation. Table 8 details two of these series in which perhaps some initial increase in virulence is shown, as heretofore the subcutaneous injection of the same dose produced death in about eight days.

TABLE 8.

SERIES I				SERIES II			
Rat	Days till Death	Chloroformed in Days	Organism Recovered in Cultures	Rat	Days till Death	Chloroformed in Days	Organism Recovered in Cultures
11.....	4	..	++	11.....	4	..	++
15.....	..	8	+	15.....	..	8	+
20.....	4	..	+++	19..... & 20.....	5 & 4	.. ..	++ +++
28–29.....	..	23	−	21..... & 22..... & 23..... & 24.....	5 & .. & .. & ..	.. 24 24 24	++ − − −

Further, the following feeding experiment shows that a few passages through guinea-pigs do not materially increase the virulence for adult rats: Each of 10 adult rats consumed 20 c.c. of a 24-hour-old sugar-free broth culture, inoculated directly from the spleen of guinea-pig 7 (Fifth passage, Series I, Table 7). They were chloroformed on the twenty-seventh day and found sterile culturally.

EXPERIMENTS ATTEMPTING TO RAISE THE VIRULENCE BY FEEDING THE ORGANS OF RATS TO RATS.

These experiments show that adult rats are not suitable for this method of passage. Out of 20 rats fed on the spleen and liver of rats dead after inoculation, 17 were chloroformed and found uninfected in from 18–36 days after the feeding. In one instance the infection was transmitted through two rats but died out in the third as shown in Table 9.

TABLE 9.

Rat	Fed on	Days till Death	Chloroformed	Cultures
2.....	(inoculated)	8	..	++
13.....	spleen and liver 2	8	..	+++
14.....	spleen and liver 13	14	..	+++
21.....	spleen and liver 14	..	22	−

FEEDING EXPERIMENTS WITH YOUNG RATS AND MICE.

Young rats (*M. norvegicus*).—Seventeen rats (50–75 gms.) were placed together in a large cage and fed on about 50 c.c. of a 24-hour-old sugar-free broth culture inoculated directly from the spleen of rat 18 (Table 5), mixed with boiled rice and rolled oats. Three of them died on the fifth day after feeding; one on the tenth day; three on the eleventh day; one on the twelfth day; one on the fourteenth day; one on the fifteenth day; i. e., 10 of the 17 were dead. Most of them showed characteristic anatomical changes post mortem. Cultures from their spleens and livers showed numerous characteristic colonies and in three instances these colonies were studied in greater detail and corresponded with the organism fed to the rats.

Further the spleens and livers of the four rats which died on the tenth and eleventh days were fed to two young rats. Both of these died on the night of the sixth day after feeding. At the post-mortem examination they both showed spleens enlarged to about four times the normal size and their spleens and livers were full of numerous yellowish-white foci of necrosis. Cultures from their spleens yielded numerous characteristic ring colonies.

A large adult male rat was fed on the body of the young rat which died on the fourteenth day. It was chloroformed in an apparently healthy condition on the sixth day after feeding and showed a typical large spleen full of yellowish granules and a much speckled liver. Of course it is possible that this rat might have subsequently recovered.

Mice (*M. musculus*).—Twenty c.c. of a 24-hour-old broth culture containing 0.1 per cent dextrose, inoculated from an agar culture from the spleen of guinea-pig 26 (Eleventh passage, Series II, Table 7) was mixed with cornmeal and fed to six mice. The mice were in one cage. One died on the eighth day, two on the twelfth day; one on the fourteenth; and one on the seventeenth day, after feeding. They showed typical spleens and livers post mortem. Litmus-lactose-agar plates from the spleen of the mouse which died on the eighth day showed hundreds of blue colonies one of which corresponded culturally with *B. pestis-caviae*. The sixth mouse was still alive but sick on the nineteenth day after the feeding.

ACTION OF THE VIRUS UPON YOUNG WHITE RATS SUCKLING AN INFECTED MOTHER.

An adult female white rat (250 gms.) with seven young rats, 10 days old, was fed on rolled oats soaked with 15 c.c. of a 24-hour-old broth culture inoculated directly from the spleen of guinea-pig 20 (Table 7) and then placed back in the cage with her young.

On the tenth day after the mother was fed one of the young rats was found in a dying condition. Four more young were found dead on the thirteenth day. Two young rats were still alive and eating nothing but their mother's milk. These two were found dead on the twentieth and twenty-second days respectively after the feeding. Three of these young rats had been partially devoured by the mother and were not examined. The remaining four showed no particular anatomical changes excepting slight congestion of the spleen. Cultures from the spleens of these four yielded typical colonies on litmus-lactose-agar plates and subcultures proved to be actively motile rods which produced gas from dextrose, but did not alter lactose nor saccharose and were completely agglutinated by the serum of a Belgian hare immunized against culture azoa rat 2 at a dilution of 1:200 in three hours at 37° C.

The mother was chloroformed in an apparently healthy condition on the twenty-second day after the virus had been fed; the mammary glands were completely atrophied: the liver was congested: the spleen was about twice the normal size, soft, and full of fine yellowish-white foci of necrosis. Cultures from the spleen and liver yielded no growth. While the presumptive evidence is that the germs reached the young through their mother's milk, one cannot rule out the possibility that the mother infected her teats after feeding on the virus. The anatomical changes clearly indicate that the mother had had a septicemic infection from which she was recovering.

SUMMARY AND CONCLUSIONS.

1. *Bacillus pestis-caviae* is an organism belonging to the group of bacteria best represented by the hog-cholera bacillus. So far as is known its original host is the guinea-pig, *Cavia Aperea*, in the spleen of which it produces pseudotuberculous nodules. It was described over 10 years ago by Theobald Smith. Culturally it is identical with

the virus of a commercial ratticide known as azoa, and with Danysz bacillus (Kràl), with a strain of *B. enteritidis* isolated from the liver of a human case, and with a strain of the hog-cholera bacillus, excepting the lack of indol production in the case of hog-cholera bacillus. Agglutination experiments show that it is identical with the virus of azoa, that it belongs to this group of bacteria and is perhaps more closely, though remotely, related to the members of this group represented by *B. enteritidis*.

2. When ingested it is acutely pathogenic for young rats (*M. norvegicus*) (50–150 gms.) and mice (*M. musculus*) which die of a septicemia accompanied by swelling and congestion of the spleen and liver, with or without the production of multiple yellowish-white foci of necrosis in these organs. The lesions in these organs often closely simulate those of plague but should not cause confusion in the naked-eye diagnosis of plague in rats: whereas hydrothorax is sometimes present in rats killed by this virus, the lungs are pale and further buboes and subcutaneous injection are never present. Bipolar-staining rods, which often occur in pairs, can be found only with difficulty in microscopic preparations from infected rats.

A large percentage of adult rats (150–350 gms.) are naturally immune to infection through the gastro-intestinal tract or when infected they subsequently recover.

3. Passing the organism by subcutaneous inoculation, through 11 or 12 guinea-pigs, does not materially increase its virulence for guinea-pigs though this method may increase its virulence to some extent for rats.

4. Adult rats are unsuitable for experimental passages by inoculation and feeding.

5. All the suckling young of an infected white rat died of the disease.

Note.—After this article had been sent in for publication I noted that the last Indian Plague Commission undoubtebly encountered this bacillus as the cause of an epizootic among their young stock guinea-pigs and also occasionally in rats. They placed it in the *B. enteritidis* group (*Jour. Hyg.*, 1908, 8, p. 306).